FRACTIONATION OF ETHYL ESTERS FROM FISH OIL

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1. Introduction

In this paper the efficiency of supercritical fluid extraction as a separation procedure will be presented, as an example the fractionation of a mixture of high boiling substances is described. Generally the product dissolved in a supercritical fluid can be separated either by increasing the temperature at constant pressure (isobaric method) or by lowering the pressure at constant temperature (isothermal method). Both phenomena can be used in fractionation procedures.

1.1 Variation of Pressure

Discontinuous variation of the pressure in either the extraction vessel or in the separator can lead to fractionation. In the first case the pressure and hence the density of the extraction agent is increased in a stepwise manner with the result that material is taken up into the supercritical phase in order of increasing boiling point or molecular weight. The dissolved material can be isolated then in either an isobaric or isothermal manner. In practice the pressure is generally raised after extraction at the lower pressure is completed.

Alternatively the extraction can be carried out at a constant pressure in the extraction vessel and the loaded supercritical phase will be expanded in a step-wise manner whereby the least volatile components are deposited first, while the better volatile components remain dissolved in the supercritical phase.

1.2 The Hot Finger

The other application of fractionation by increasing the temperature is shown in Figure 1. The extraction vessel has been combined with a rectification column consisting of a pressure tube packed with stainless steel packings. A heated head of column, the so-called Hot Finger, is fitted onto the top of the column and is held at a higher temperature than the rest of the apparatus. On contact with the Hot Finger the density of the supercritical loaded phase decreases and the less volatile components condense and drop back into the column and are subjected to rectification in the same manner as in a conventional distillation. The product still dissolved in the supercritical phase after passing the Hot Finger is isolated by decreasing the pressure as already described.

2. Fractionation of Fatty Acid Ethyl Esters

As an example of various applications which are investigated at the Max-Planck-Institut für Kohlenforschung in Mülheim-Ruhr in this paper the fractionation of a mixture of ethyl esters of fatty acids is

described. This investigation was concerned with the separation of eicospentaenoic acid (EPA) out of a mixture of fatty acid ethyl esters from codfish oil. The starting material consisted of ethyl esters of saturated and unsaturated fatty acids from C(14) to C(22) partly containing up to 6 double bonds. In order of the high boiling points of these esters a conventional vacuum distillation needs relatively high temperatures so that, first, decomposition cannot be excluded and, second, a separation into single components is hardly possible.

In co-operation with the NIPPON SUISAN KAISHA,LTD - TOKYO (Japan) it was tried to fractionate such a mixture, the composition is shown in table 1, by using supercritical carbon dioxide to isolate a C(20-5)-rich fraction. (x-y) means the ethyl ester of a fatty acid with x carbon atoms and y double bonds.

Table 1 Composition of the Codfish Oil

Acid	%	Acid	%
14-0	5.80	20-1	11.43
15-0	0.19	20-4	0.50
16-0	12.88	20-5	14.46
16-1	9.79	22-1	8.64
18-0	2.66	22-4	0.43
18-1	23.25	22-5	0.49
18-2	0.16	22-6	5.74
18-4	2.19		

In a series of experiments carried out in an equipment similar to that shown in figure 1 various conditions were investigated to optimize this procedure, mainly the following:

1. The increase of the temperature of the Hot Finger in order to increase the the reflux ratio.
2. The increase of the pressure to get a higher loading of the supercritical phase.
3. Variation of the length of the column and of the packings

In all these experiments the temperature of the extraction vessel, column and separator was kept constant at 50°C, the supercritical loaded phase was expanded to 25 bar in the separator and the flow rate of the supercritical carbon dioxide amounted to about 25 l/h. Samples of the loaded supercritical phase after passing the Hot Finger were analyzed each hour by gaschromatography.

2.1 Variation of the Temperature of the Hot Finger

2.11 Without the Hot Finger

The first experiment was carried out without the influence of the Hot Finger that means, the temperature of the Hot Finger was kept also at 50°C. The starting material was divided into 10 fractions by SCF extraction with supercritical carbon dioxide. It shows that no separation comes off, only an enrichment of the lower boiling esters C(14) and C(16) in the first fractions and of C(20) and C(22) in the later fractions according to their boiling points.

2.12 With the Hot Finger

The variation of the temperature of the Hot Finger really led to an increase of the reflux ratio but the selectivity decreased by exceeding the temperature of 90°C.

The curves in figure 2 show the difference in selectivity at the two temperatures 90 and 100°C. Here out of the whole mixture only the content of two components in the supercritical loaded phase, that are the sum of C(16)- and C(20)-esters analyzed by GC, are plotted versus the extraction time. To get a high purity combined with a yield as high as possible at 90°C one is able to collect a fraction amounting to 13.5 % of the starting material containing 50 % of the C(20)-esters of the feed-stock with a purity of 96.2 %. At 100°C it is only possible to collect 25.7 % of the C(20)-esters with a maximum concentration of 85.5 %.

2.2 Variation of Pressure

The loading of the supercritical carbon dioxide depends on the pressure of the system. Under constant conditions of temperature and flow rate of the supercritical carbon dioxide the increase of the loading with raising the pressure can be demonstrated by the extraction rate: at 150 bar 15 g/h extract could be isolated, at 170 bar the amount was 55 g/h and at 200 bar 140 g/h. But the higher extraction rate was accompanied by a decrease in selectivity, at 200 bar no fractionation could be realized.

2.3 Results of Optimization

The results of these optimization experiments suitable only for the equipment we used are listed in table 2.

Table 2 Conditions and Results of Optimization

Nr	Temperature Hot Finger (°C)	Pressure (bar)	Rate of Extraction (g/h)	Max. Content (%) *)	50 % Yield C(20)-esters Extract (%) **)	Purity (%)
01	80	120	8.2	84.2	17.2	75.0
02 ***)	80	120	13.1	92.2	14.4	86.8
03 ****)	80	120	12.8	55.6	25.1	50.3
04	90	200	138.5	no fractionation		
05	90	170	55.3	no fractionation		
06	**90**	**150**	**14.7**	**98.9**	**13.5**	**96.2**
07	120	200	38.2	45.3	42.8	33.0
08	120	170	12.4	89.3	15.7	80.7
09	120	150	7.8			
10	100	150	15.8	92.7	14.7	84.5

*) of C(20)-esters in the loaded supercritical phase (without CO_2)
**) related to the starting material
***) with smaller packings
****) without packings

In all these experiments the extraction temperature, the length of the column and the flow rate of the supercritical carbon dioxide were kept constant. Besides the reaction conditions in this table the results of the fractionation are listed in relation to the isolation of the sum of C(20)-ester.s In this table the maximum content means the maximum concentration of C(20)-esters in the supercritical loaded phase during the extraction, analyzed by GC. Next to the last column of the table shows the amount of extract including 50 % of the C(20)-esters,s related to the starting material. The last column shows purity of this fraction.

The best result was obtained in the experiment 06 with a temperature of the Hot Finger of 90°C and a pressure of 150 bar. Both at higher temperatures and higher pressures the selectivity of the fractionation decreases.

2.4 Two-step Extraction

The last experiments have shown that it is impossible to get a higher yield than 50 % with a purity of more than 90 %. Under the best conditions described above it should be tried to raise the yield of C(20)-esters by a two-step extraction procedure.

2.41 First Fractionation Step

In this experiment the codfish oil was extracted with supercritical carbon dioxide and by means of the hourly GC-analyses of the loaded supercritical phase several fractions were collected. In the first fraction all extracts were joined in which no C(20)-esters could be detected. In the second fraction the extracts up to 10 % C(20)-esters were collected and in the third fraction the extracts which contain the last traces of C(16)-esters. The next fraction contained only C(18)- and C(20)-esters of different composition. After the content of C(20)-esters of more than 90 % in the supercritical phase was reached the main fraction was collected until the content again reached 90 % after passing a maximum content of about 99 %. The next fraction consisted only of C(20)- and C(22)-esters in various combinations. When the value of the C(20)-esters was lower than 2 %, then the rest of the inserted material was extracted without using the Hot Finger. This last fraction besides C(22)-esters contained some not identified higher molecular components. Figure 3 shows the fractionation curves where the composition of the mixture dissolved in the supercritical phase is plotted versus the extraction time respectively versus the amount of extract in percent of the starting material.

The main fraction contains 48.2 % of the C(20)-esters which have been containing in the original starting material with a purity of 95.8 %, this is the first part of the desired product.

2.42 Second Fractionation Step

The fractions got in the first fractionation step containing only C(18)- and C(20)-esters respectively C(20)- and C(22)-esters were combined to give the new starting material for the second fractionation step. The first and the last fraction which contained only C(14)- to C(18)-esters respectively C(22)-esters were rejected.

Fraction 2 with the highest concentration of C(18)-esters was stored separate. This new starting material amounted of 24.5 % of inserted material of the first fractionation step. The composition, analyzed by gaschromatography, was as followed:

C(16)-esters	traces
C(18)-esters	23.56 %
C(20)-esters	45.28 %
C(22)-esters	28.74 %

This mixture was fractionated into 5 fractions under the same conditions as used in the first step. In table 3 the results of the GC-analyses are listed.

Table 3 Composition of the Fractions of the Second Step

Nr	% Extract	% C14	% C16	% C18	% C20	% C22
01	25.5	----	1.3	76.1	20.8	----
02	17.3	----	----	25.3	73.3	----
03	**21.2**	**----**	**----**	**1.6**	**97.5**	**0.6**
04	10.8	----	----	----	48.6	51.3
05	24.3	----	----	----	0.6	98.0

In this fractionation step it was impossible to get a fraction without C(20)-esters. Therefore the first fraction was collected until the content in the supercritical phase reached about 50 %, in this fraction the main portion of C(18)-esters was found. Fraction 3 was collected in the same manner as the main fraction in the first step, herein all extracts were combined in which the content of C(20)-esters was higher than 90 %. The last fraction, a very clean C(22)-ester mixture, was extracted without the Hot Finger.

Fraction 3 consists of 45.7 % of the C(20)-esters in the starting material of the second step, respectively about 20 % related to the original Codfish oil with a purity of 97.5 %. If fraction 2 and 3 are mixed the yield of C(20)-esters raises up to 73.8 % in course of which the purity decreases to 86.6 %.

2.43 Results of the Two-step Fractionation

If the main fraction from the first step and fraction 3 from the second are mixed one gets a portion of extract which contains 67.7 % of the C(20)-esters in the original ester mixture with a very high purity of 96.3 %. Including fraction 2 from the second step, the yield increases to 80 % with the sufficient purity of 92 %. This mixture contains 77 % of the C(20-5)-ester of the inserted codfish oil.

3. Conclusions

It could be demonstrated that it is possible to separate a mixture of high boiling fatty acid ethyl esters into single components by fractionation using supercritical carbon dioxide at an extraction temperature of 50 °C. Regarding the areas of the single C-numbers a rather sharp separation could be achieved. In the case of the C(20)-esters the best results were obtained but also the C(18)-esters and the C(22)-esters could be isolated with a high purity.

In the experiments described the fractions containing only C(18)- and C(22)-esters besides the desired C(20)-esters were fractionated separately in a second extraction step. The better procedure would be to recycle this portion to the starting material to enrich the concentration of the desired esters in order to increase the yield of these esters in one extraction step. Such a procedure could be carried out in the following manner: In a dicontinuous process the fractions containing only C(14)- and C(16)-esters respectively C(22)-esters were rejected, the fraction with the highest concentration of the desired esters would be the final product and all other fractions would be recycled to the process.

The main assignment of these investigations was to separate most of the C(20)-esters in high yield and high purity. It seems that it is possible to enrich the C(20-5)-ester by a suitable fractionation. But these experiments have also shown that it will be rather difficult to achieve a clean separation of C(18-1)-, C(20-5)- and C(20-1)-esters because of their similarity, the C(20-5)- and the C(20-1)-esters differ only in 8 H-atoms, i.e. 2.4 % in molecular weight related to the molecular weigth of 332.6 for eicosapentaenoic acid ethyl ester.

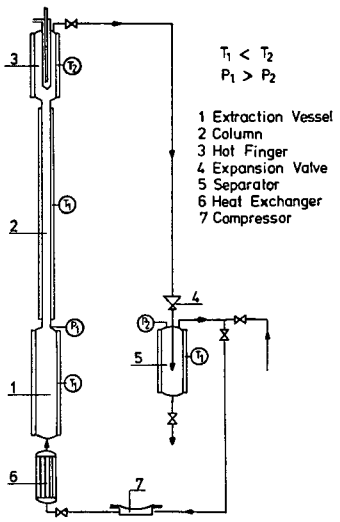

Figure 1 Fractionation Apparatus with Hot Finger

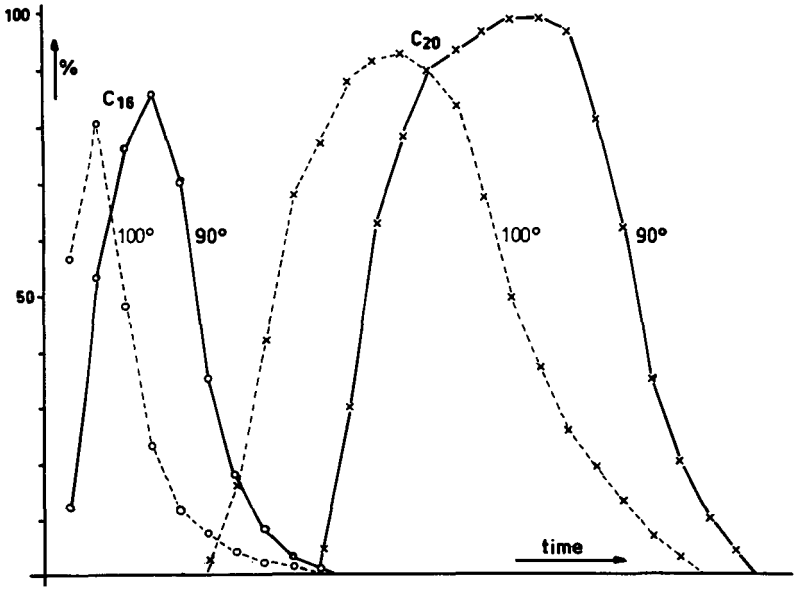

Figure 2 Separation Curves at 90 and 100°C

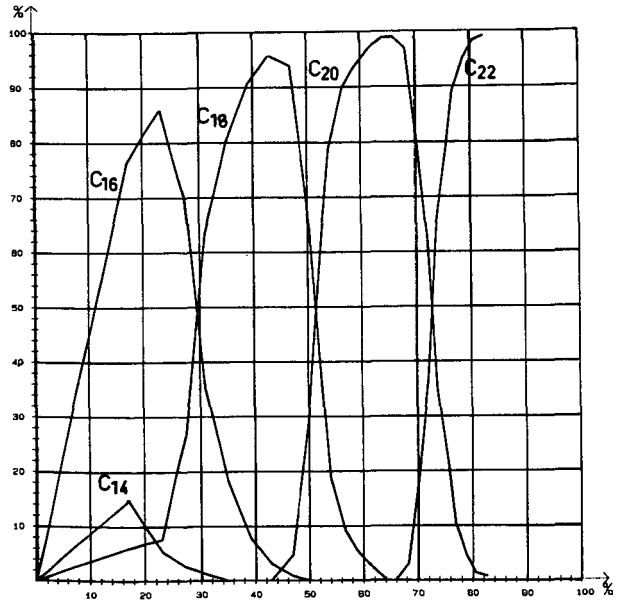

Figure 3 Separation Curves (Composition in Supercritical CO_2)